THE FUNCTIONAL ORGANIZATION OF THE NERVOUS SYSTEM OF ENTEROPNEUSTA

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INTRODUCTION

The chordate affinities of balanoglossids have been widely accepted ever since Bateson advanced the suggestion in 1885 and have not seriously been challenged since Spengel (see especially 1893). Some of the most characteristic features of chordates relate to the nervous system. Moreover, the evolution of that group has outstandingly involved this system. It is of considerable interest, therefore, to inquire into the nature of the nervous mechanism of the Enteropneusta. Investigations of a purely morphological type were made by the earliest workers, but we lack, as yet, any conception of the functional stage of development represented or the plan of organization. The purpose of the present paper is to report some simple experimental evidence concerning the physiologic pattern of the nervous system in the Enteropneusta, and to offer a tentative generalized picture of its condition.

The intimate relation between receptors, nervous mechanism, and effectors renders it appropriate, in the case of animals whose general behavior is unfamiliar, to consider all three systems as an integrated unit. Since our only knowledge of the activities of the first two named systems is gained through observations of the overt activities of the effectors, the latter system is treated first.

The original observations contained herein relate chiefly to two eastern Pacific species, *Saccoglossus pusillus* (Ritter) (formerly *Dolichoglossus*) and *Balanoglossus occidentalis* (as yet a manuscript species). These were collected near San Diego, California, and the majority of the experiments were carried out in the laboratories of the Scripps Institution of Oceanography. It is a pleasure to acknowledge the kindnesses extended to me by Dr. H. U. Sverdrup and Dr. M. C. Sargent of that Institution.

EFFECTORS AND THE CHARACTER OF RESPONSE

The behavior of enteropneusts originates in at least three effector systems: ciliary, glandular, and muscular.

Cilia

Balanoglossids exhibit an extraordinarily high level of development of the general ciliature, and of dependence thereon. The entire surface of the body, external and internal (with very limited local exceptions), is provided with cilia which beat, in all probability, essentially continuously throughout life. Such an elaborate and extensive mechanism might be expected to perform more than such localized functions as the creation of respiratory or nutritive currents.

Removed from their normal habitat and placed in an aquarium for observation, specimens of *Saccoglossus pusillus* employ their cilia as the principal locomotor organs! True, parts of the body move, muscularly, relative to other parts; and progression may be accomplished by a series of "inch-worm" like movements involving extension and fixation of the proboscis followed by contraction of the trunk. But this does not occur regularly (Benham, 1899, describes it in *S. otagoensis* but does not indicate how usual it is), nor does any snake-like pushing against minute obstructions in the substratum. Peristaltic progression may be observed, but, under these artificial conditions at least, slow progress of the whole animal by means of cilia represents the chief method of locomotion.

Cilia are also used in building sand tubes and in burrowing. They can be directly observed in the former process. In *S. pusillus*, as Ritter and Davis (1904) and I have observed it, the proboscis cilia play the dominant rôle. The proboscis exhibits continual exploratory movements, its tip touching the substratum now here, now there. Every sand grain adhering to the sticky mucus thereon immediately begins to move backwards over the surface of the organ. Thus develops a stream of rapidly moving particles which, however, stop their advance at the level of the collar; here they accumulate to form a short sheath. By the force of the continual stream of particles from the powerful proboscis cilia, the sheath is pushed back, eventually to cover the entire trunk. The spectacular nature and the speed of this process not only emphasize the importance (mentioned below) of a sand tube but also suggest the probable rôle of the cilia in burrowing. For, doubtless, these organelles importantly contribute in this process, especially in the Ptychoderidae and Harrimaniidae where the extensor muscles of the proboscis are even more poorly developed than in the Spengeliidae (van der Horst, 1927–39).

Other functions of the cilia are entirely conjectural. Since not even a plausible guess has been offered concerning the function of the gill slits in balanoglossids, the significance of the highly developed ciliature lining those passages remains unknown. Whether the cilia

are of importance in an exchange of fluids between the coelomic cavities and the ocean through the proboscis and collar pores remains, with the antecedent question of the existence of such exchange, unproven. Notable as the only experimental evidence on the question is Bateson's (1885) demonstration that particles injected into the proboscis or collar coelom are ejected through the respective pores; but he found no intake of water, such as has frequently been thought to dilate the proboscis in the cycle of digging movements (e.g. Spengel, 1893).

Are the cilia of balanoglossids under nervous control? No work has been specifically directed at this point, but some observations are suggestive. The cilia beat, as would be expected, in the absence of nervous function, thus in anaesthetized animals and in the embryo before a nervous system has appeared. That is, ciliary activity is not dependent on the nervous system, a property generally ascribed to all cilia. The question which remains is: Can the nervous system modify the activity of the cilia? Since Parker's dictum in 1919, ". . . in fact, it may be stated that at present there is not the least ground for the assumption that true nervous activity is in any direct way involved in ordinary ciliary reactions," [1] there has been brought forward convincing evidence of nervous modification of ciliary activity in several cases (Göthlin, 1920; Alverdes, 1922; Copeland, 1922; Merton, 1923; Carter, 1926; McDonald, Leisure and Lenneman, 1928; Seo, 1931). It would seem from the evidence available that, over and above the intrinsic, epithelio-genic beat and coördination of all ciliary fields, some ciliary fields have come under nervous control. In such epithelia the normally continuous beat may be stopped, started, reversed or otherwise modified over the whole or only part of the field, at the "will" of the nervous system. Carter (1926) has set forth a rigid series of specifications for the thoroughly satisfactory demonstration of a case of nervously regulated cilia. No one has sought to satisfy these criteria for the balanoglossids, but, doubtless, many have observed, as I have, the striking behavior of the epithelium of adult enteropneusts in life. Here is seen an impressive degree of ciliary reversal and inhibition. Not whole fields but very small areas stop beating and start again many times in a few seconds, or only occasionally. Localized changes in direction of beat likewise occur. Now considerable, now limited areas of epithelium beat, now dorsally, now obliquely posteroventrally, dorsally again, anteriorly, anteroventrally, ventrally, shifting in rapid succession, the environment meanwhile remaining to the human senses uniform. Such remarkable behavior is difficult to explain,

[1] The qualification "ordinary" is puzzling. Nowhere in Parker's subsequent discussion does he refer to any extraordinary ciliary activity that might form an exception to his generalization.

in the absence of external stimuli, on the basis of an intrinsic epithelial mechanism acting entirely independently, but, of course, additional evidence is desirable before a nervous control is assumed. These features of ciliary activity were observed in *Saccoglossus pusillus* and only on the surface of the trunk, the proboscis cilia beating steadily posteriad.

It would seem probable, then, that the unusually extensive and elaborate ciliary mechanism of balanoglossids is of profound importance to the economy of the animal, at least in certain kinds of locomotion, in sand tube formation and burrowing. The occurrence of specialized local ciliary apparatus and the complex behavior of certain ciliary fields indicate that it is probably important in other more specific and complex functions. The occurrence, speed, and localization of inhibition and resumption as well as reversal of ciliary beat suggest that the present animals may offer a new case of nervous control of cilia.

Glandular Activity

Enteropneusts are among the most copious producers of mucus known. Furthermore, to reason from the variety of histologically differentiated types of unicellular epithelial glands, they must elaborate a considerable array of different substances. The nature, importance, and modifications in secretion of these substances remain virtually unknown. From the highly developed state of the mechanism, the volume of its production and the habit of the animals, it seems safe to regard this system as of great importance. But no evidence can be cited bearing on the question of control of glandular activity beyond the facts that anaesthetized worms and isolated strips of epithelium continue to secrete. Probably largely independent of the nervous system, the existence or nature of direct or indirect nervous control cannot as yet be surmised.

Light Production

Several species of balanoglossids have been reported to produce light upon stimulation after dark-adaptation (Kuwano, 1902; Crozier, 1917). The nature of the phenomenon has not been determined in these animals. Crozier assumes that it is intrinsic, i.e., that the light is produced by the worm itself, and it may be that some of the plethora of unicellular integumentary glands are responsible. In this case, of course, the phosphorescent organs should not be listed as a distinct effector system. The only facts known about the phenomenon are that it is elicited by external stimuli such as poking the worm or tapping the aquarium, that it is inhibited by exposure of the subject to light,

and that it is accomplished as well by small fragments as by intact animals. Regarding the question of nervous control, Crozier argues from the last-named fact that light production must be independent of the nervous system. But, in view of the high degree of neuromuscular autonomy of even small fragments, demonstrated later in this paper, his position is no longer tenable, and the first two facts named above suggest reflexes involving the nervous system.

Muscular Activity

The normal habit of life of all enteropneusts is doubtless benthonic. Many species are burrowers. Their food habits though not yet elucidated are clearly passive, for in general these animals are sand-eaters and obtain their nourishment from organic matter contained in the substratum. Their means of protection, as well, seem likely to prove passive, though even less understood than the means of nutrition. Very possibly the well-known iodoform odor of many species functions in this connection and perhaps we may thus account for some of the diverse integumentary glands.

Corresponding to such a passive mode of life, balanoglossids are among the most sluggish of all free-living animals. Not only are their movements characteristically sluggish but the observer is struck by the small number of different responses possible. The movements incident to burrowing and to advance and retreat within the burrows, comprise the sum total of muscular exertions of most of these animals.

Cases of an extraordinary degree of activity for enteropneusts are the reports of Ikeda (1908) and Spengel (1909) of the pelagic occurrence of two species of the genus *Glandiceps*. Swarms of individuals were encountered swimming at the surface under conditions which could not be correlated with breeding phenomena or any other obvious factor. The mechanism of swimming is not made clear.

In view of the frequent misconceptions thereof, a word may be said concerning the mechanism of burrowing. Most importantly, it is not yet clearly understood, even in essential respects. The use of the coelomic pores for dilation and collapse of the proboscis and collar with sea water has not been demonstrated and is, on several anatomical grounds, unlikely. The service of the "notochord" and its secreted skeletal sheath is principally that of the muscle attachment, secondly that of strengthening the proboscis-collar junction; but it is not to be thought of as providing an otherwise flabby proboscis with the rigidity required for digging in heavy mud and sand. For that organ, in short- as well as in long-proboscis species, *is* flabby, and balanoglossids burrow slowly and inefficiently because of their lack of rigidity. The muscles principally responsible for forcing the proboscis into the sub-

stratum, i.e., the extensors of that organ, are the circular muscles which are conspicuously poorly developed in all species, and especially so in the Harrimaniidae and Ptychoderidae. Much more voluminous are the longitudinal muscles which shorten and, incidentally, swell the proboscis. (The disproportion is even greater in collar and trunk.) These facts emphasize the probabilities that: (1) those balanoglossids called, above, "burrowers" are chiefly livers *in* (more or less permanent) burrows, not active diggers, and (2) cilia are very likely of great importance in burrowing, as in sand-tube formation (very likely in the same way) and in locomotion.

To emphasize the paucity of clearly-defined and diversified movements, the results of the search for responses lending themselves to physiologic testing in the experiments described below may be cited. Using *Saccoglossus pusillus*, I was able to find but a single reflex even approaching an adequately dependable and clean-cut piece of behavior for which to test experimental subjects. This is elicited by tactile stimulation, for example, a gentle poke with a probe, applied to the proboscis. If each of a series of conditions having to do with the state of the specimen has been satisfied, such a stimulus results in the most rapid muscular response this animal is capable of, namely, a contraction of the longitudinal muscles of the trunk. The trunk is thereby shortened and coiled, but the collar and proboscis need not be involved in the movement at all.

The predictability of this "shortening reflex" depends most on the following conditions. The specimen must be in good condition, uninjured in collection except as regards the abdomen, which may even be missing without affecting the response. It must have been but recently removed from its burrow, whether in mud flat or aquarium. It should be active, moving about the vessel in exploratory fashion; the best responses are obtained from animals stretched out straight, in forward, doubtless ciliary, progression. And, finally, it must not have executed a generalized contraction within the preceding five minutes.

This is, in all probability, a true nervous response, since it takes place at a distance from the point of stimulation and is abolished, reversibly, by light anaesthesia such as does not impair ciliary or glandular activity or irritability of the muscles to strong direct stimulation.

We have here to do with a motor apparatus consisting of smooth fibers, not segregated into discrete muscles but occurring in loose masses or layers. It is functionally characterized by sluggish action, generalized responses, a lack of differentiated reflexes, and a relatively simple rôle in a rather passive, benthonic habit of life.

Receptors and the Known Adequate Stimuli

Although several authors have suggested a sensory nature for certain peculiar structures of the integument (Spengel, 1893; Assheton, 1908; van der Horst, 1930; Brambell and Cole, 1939), the lack of adequate reason for so regarding them makes it necessary to say that as yet no sense *organ* has been demonstrated in the Enteropneusta. This is a very astonishing fact, considering the early origin of differentiated organs of reception, and indicates not only a very simple type of receptor mechanism, but the probability of a very generalized type of irritability. The receptors are assumed to be scattered sense cells in the epithelium. Only one author has claimed to have seen sensory cells in balanoglossids, Hess (1938) having identified certain large basiepithelial, bipolar cells as photoreceptors.

The facts known about sensory physiology in enteropneusts fall into three groups according to the nature of the stimuli: mechanical, chemical, and photic.

Mechanical Stimuli

The irritability to probing and to tapping of the aquarium has already been mentioned. Characteristic responses, varying with the species, result from the stimulus of contact with a sandy or muddy substratum.[2] Species-characteristic behavior in response to exposure by the tide is also reported. This involves retraction or protrusion of the proboscis, or migration up or down in the burrow (Ritter and Davis, 1904; Morgan, 1894; Assheton, 1908; Stiasny, 1910). Disturbance of the substratum, as by footfalls, brings about quick retraction in the burrow.

Chemical Stimuli

We know virtually nothing concerning the sensitivity of balanoglossids to dissolved chemicals. The single report on record is the statement of Crozier (1915) that "for the chlorides of the alkaline metals, the normal lyotropic series, $K > NH_4 > Li > Na$, was found to express their stimulating efficiency; this was mainly a kation stimulation, but in the case of salts ($CaCl_2$, e.g.) which did not stimulate strongly, other anions (in this case $Ca(NO_3)_2$) were effective as stimulating agents." Crozier, further, was able to separate, by exhaustion or anaesthesia, the mechanical-chemical irritability from photic sensi-

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[2] The curious response of *Saccoglossus pusillus* to the absence of mud is death, within as short a time as two or three days. Whether this is due to starvation, exhaustion or some other factor has not been determined, but the suddenness of death and the rapidity of cytolytic disintegration even in well-aerated, darkened aquaria are spectacular.

tivity, but the two former types of irritability could not be physiologically separated. He concluded that there are generalized receptors open to both mechanical and chemical stimulation, but recognized that these facts are "insufficient proof of the separateness of the sensory organs concerned in the reception of photic stimulation." He felt, however, that they do show "that photic irritability depends upon a process distinct from that implicated in the other modes of stimulation."

Photic Stimulation

Crozier noted in 1915 that *Ptychodera* and *Glossobalanus* showed "differential sensitivity" to light, and were indeed negatively photokinetic. Pursuing this form of experimentation, he found (1915, 1917) that: (1) The sensitivity to light was enhanced by dark-adaptation. (2) Photic and other forms of irritability could be readily separated physiologically (see above). (3) The collar nerve cord is not necessary for the kinetic response to light. (4) Posterior fragments or pieces of abdomen, including the caudal tip, are able to respond, but move away from the source of light tail first! (5) The tip of the proboscis is most sensitive, although the whole body exhibits some irritability. (6) Exposure to light greatly inhibits the ability of these animals to produce light, though such production could always be brought about by moderate induction shocks. (7) Even small fragments are able to produce light and are inhibited therefrom by exposure to light.

The only other experimental work upon enteropneusts has been that of Hess, whose interest was primarily in the photic response of these animals. Hess (1931, 1936, 1937, 1938) confirmed the observations of Crozier and then attempted to analyze the response by removing various regions of the body. He reported that the response (orientation and movement away from the source of light) was unimpaired by cutting off the proboscis, or the dorsal third of the collar (to remove the collar nerve cord), or its ventral third (to remove the "ventral nerve cord" of the collar [a cord which does not exist]), or the trunk and proboscis (leaving only collar and "peduncle" or proboscis stalk). The isolated proboscis, likewise, was able to respond. But, pieces from which the proboscis and its peduncle had been removed (or proboscis, peduncle, and collar, i.e., isolated trunk fragments) were unable to orient with respect to light. He concludes that the peduncle contains a "nervous center" concerned with light reactions, a center of which the proboscis, unlike the rest of the body, is independent. In *Ptychodera bahamensis* Hess considered the reactions sufficiently clean-cut to take reaction times. For all regions or combinations except the isolated proboscis these times were very nearly the same (1.90″–2.77″),

including the, presumably, optimal combinations—peduncle, collar, and trunk—as well as abdominal fragments which cannot orient but only, it is to be inferred, squirm aimlessly. The reaction time of the proboscis is given as 25.23″. This remarkably slow result Hess explains as being due to the fact that the proboscis has now been severed from the "central nervous system." That this "most sensitive region of the body," which can, when isolated, orient and move away from light, should lack this nervous ability—a rapid light response—which is possessed by a trunk fragment, is difficult to believe. His interpretation (or the figure for the reaction time of the proboscis) seems the more dubious (1) in view of the concept, arrived at below, of the nature of the "central nervous system," (2) in view of the independence of the light response of the great nervous concentrations in any case (Crozier, 1917, and below), and (3) in view of the general behavior and properties of the proboscis.

In 1938 Hess repeated on *Saccoglossus kowalevskii* those experiments involving intact animals. Again, intensities of light above a certain minimum resulted in negative orientation with little tendency to trial and error movements; but weak illumination produced a tendency to orient toward the source. Pin-point light exploration showed all regions to be sensitive, but some regions more so than others, the proboscis being the most sensitive.

My own experiments with photic stimulation were performed on *S. pusillus*. This species was found to be definitely negatively tropic although its response was very soon lost after removal of the animals from mud and exposure to light. The photic response is abolished by light anaesthesia, but returns after brief exposure to clean sea water. This shows, following Parker (1919), that the reaction depends upon the nervous system.

The methods of investigation used by Hess on *Ptychodera* presuppose an extended, straight animal, relatively motionless before exposure to light, capable of turning promptly upon exposure in a definite direction and without trial and error or "indecision." *S. pusillus* rarely assumes any but a coiled position with only the proboscis relatively straight, almost continuously moves about in exploratory fashion if fresh and healthy, and responds, often (especially in specimens removed from mud for some time, as in operatives and their controls which are rested in a constant temperature darkroom for at least one hour) with considerable hesitation. These differences render inapplicable to *S. pusillus* the technics of Hess, especially the determination of reaction times. But the existence of photic irritability was clear and the regional assignment of responsibility for the negative orientation to light was quite possible.

Hess's (1937) method of analysis was repeated upon variously constituted fragments of the body. After operation these fragments were dark-adapted at 20° C. for 1 to 2 hours and then exposed to a laterally incident beam of light with the following results. (1) Isolated proboscides, cut well forward of the peduncle, can regularly turn away from the source of light. (2) As would be expected, fragments consisting of proboscis and peduncle or of proboscis, peduncle, and collar readily respond and orient themselves in the same way. (3) Specimens with the collar mutilated, either by cutting across or removing the entire dorsal third (to include the collar nerve cord) are perfectly able to orient with respect to light. Controls with the ventral third of the collar missing or transected respond similarly. (4) Fragments of trunk region are incapable of orientation with respect to the direction of illumination. (5) Pieces consisting of collar and trunk but without peduncle or proboscis are incapable of orientation. Thus far all results correspond exactly with those of Hess. But (6) pieces similar to the last-named with the addition of the peduncle, i.e., with nothing removed but the proboscis, never succeeded in turning away from the light source in these experiments. The operations in this case were carefully performed so as to leave intact the entire peduncle and base of the proboscis, since this result is diametrically opposed to that reported by Hess for *Ptychodera*. It was found, by depriving further specimens of *Saccoglossus* of various fractions of the proboscis, that nearly one-third of its length must remain attached to the peduncle to obtain occasional definite responses, and more than one-third for consistent negative phototropic movements. It must be recalled that *Saccoglossus* is characterized by an extremely long proboscis; when two-thirds of it has been removed the remainder still exceeds the collar in length and is, proportionally, longer than the intact proboscis of *Ptychodera*. The long proboscis permits that much more exact delimitation and transection. The present conclusions, therefore, may be stated thus: although all regions of the body are sensitive to light, responding by aimless squirming, only the proboscis is capable of orienting with respect to the source of light, and the normal response of the intact animal is dependent upon that organ. The assumptions of a "center" in the usual neurologic sense, or of a central nervous system are not justified by the evidence at hand. Hess's results are open to the same interpretation, if it be supposed that his operation corresponding to (6), above, left a minimal amount of the (very short) proboscis still attached to its peduncle to orient the preparation to light.

The general nature of the sensory physiology of balanoglossids is,

therefore, clear, though the details are only sketchily indicated. Generalized receptor cells are scattered throughout the integument though differentially concentrated. The proboscis is most sensitive and, in a general way, a gradient of decreasing sensitivity from anterior to posterior exists. The senses of enteropneusts are at a very low level of development and it would seem probable that the animals are not greatly dependent on them. This condition may be correlated with the essentially passive, bottom-living habit of life. Though sensitive to a variety of forms of stimulation, the few and generalized
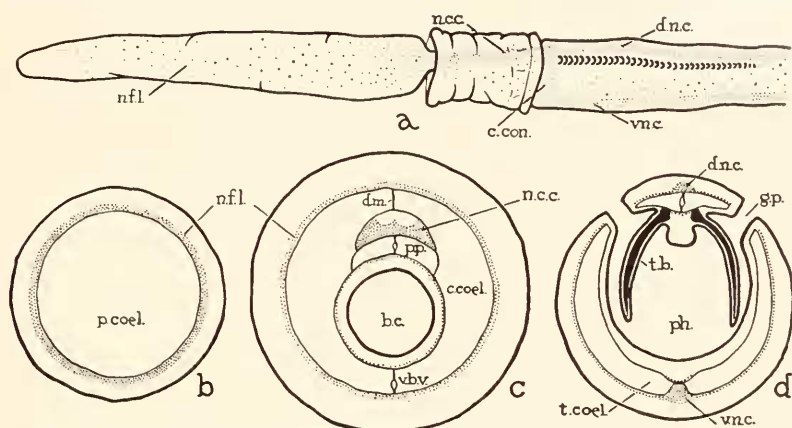

Fig. 1. Schematic representation of the nervous system of *Saccoglossus pusillus*. The nerve fiber layer of the epithelial nervous system is indicated by stippling. (*a*) Lateral view of proboscis, collar, and anterior end of trunk. (*b*) Transverse section of proboscis, (*c*) of collar, (*d*) of pharyngeal region of trunk. Organs occupying coelom have been omitted. *b.c.*, buccal cavity; *c.coel.*, collar coelom; *c.con.*, circular nerve connective; *d.m.*, dorsal mesentery; *d.n.c.*, dorsal nerve cord of trunk; *g.p.*, gill pore; *n.c.c.*, internal nerve cord of collar; *n.f.l.*, nerve fiber layer of superficial plexus; *p.coel.*, proboscis coelom; *ph.*, pharynx; *p.p.*, perihaemal pocket of trunk coelom; *t.b.*, tongue bar (coelom not shown); *t.coel.*, trunk coelom; *v.b.v.*, ventral blood vessel; *v.n.c.*, ventral nerve cord of trunk.

kinds of responses suggest a low order of differential recognition of stimuli, and little or no differentiation of "specific nerve energies." The beginnings of complexity are seen, for instance, in the dependence of the whole upon one part for orientation to light, but on the whole the level that has been achieved in this group is lower than that of many of the coelenterates—the simplest animals having any nervous system.

Functional Organization of the Nervous System

Anatomically, the nervous system of balanoglossids, as described by its discoverer (Spengel, 1877, 1893, and not since significantly modified), consists (Fig. 1) of a general superficial plexus located at the

base of the outer epithelium everywhere over the body and locally thickened to form so-called cords in the mid-dorsal and mid-ventral lines of the trunk—through the dorsal coelom of the collar as the only internal part, and again on the surface of the peduncle. All of these regions of nervous concentration, except the collar cord, are continuous with and connected by the general plexus; thus the peduncular thickening and the proboscis plexus, the dorsal and ventral trunk cords and the lateral trunk plexus are continuous basiepithelial layers. The ventral cord is confined to the trunk and at its anterior end a circular connective, traversing the collar-trunk boundary, passes from it to the dorsal cord where that is continued into the collar coelom.

The most important feature of the neuromuscular organization of these animals, and one profoundly conditioning the character and possibilities of experiment on it, has already been emphasized, namely, the simplicity and lack of variety of responses. Not only this, which could be said of an animal with a single, clean-cut, invariable response, but the responses of *Saccoglossus pusillus* are not uniform and dependable. The single sufficiently predictable reflex found in this species has been described above as the "shortening reflex."

The Nerve Cords

If the dorsal nerve cord of the trunk in a healthy specimen of *S. pusillus* be transected a little behind the collar (the technic of the operation and the extent of the cut make virtually no difference; a single stroke with sharp iridectomy scissors was found most convenient), a preparation is obtained in which the shortening reflex is hardly impaired. Often it is less certain, sometimes slower, but many responses are obtained which cannot be distinguished from those of normal subjects. Using *Balanoglossus occidentalis*, a large species only occasionally obtainable, this result is even clearer. It must be said that all experiments with this species were performed upon larger or smaller fragments, since I have never seen an intact specimen. However, it is easily demonstrable with other species, and has indeed been well known to earlier workers, that loss of the posterior part of the trunk has no noticeable effect on behavior. With this species it is possible to show, not only a nearly normal posterior response to anterior stimulation, but also a proboscis response to trunk stimulation virtually unaffected by transection of the dorsal nerve cord.

Similar operations affecting the ventral cord, however, have a marked effect on the shortening reflex. It is greatly impaired posterior to the level of the cut, is slower, prolonged as sluggish writhings, and has a higher threshold of stimulation. Again, the results with

Balanoglossus are even more clean cut. The animal ceases its spontaneous exploratory movements, but spontaneous peristaltic waves pass back from the level of the cut, as in isolated trunk fragments.

The effect of transection of both cords is even more pronounced. Now the response is virtually abolished posterior to the level of the operation. Stimulation adequate for the controls is entirely ineffective. Stronger prodding of the proboscis may elicit contraction of proboscis, collar, and trunk muscles anterior to the cuts, and a few of the contiguous muscles immediately posterior thereto. Repeated, relatively strong stimulation does, however, invariably produce movements of the abdomen, although not of the same character as the normal rapid shortening.

Lateral incisions, in the frontal plane, at the junction of trunk and collar, one on each side, yield results similar to the ventral cord transection above. Such incisions interrupt the continuity of circular connectives which thus appear to be of considerable importance in normal communication between the proboscis and the ventral cord of the trunk; that is, the alternative path through the general lateral plexus is not the route normally employed in rapid long distance reflexes. If the dorsal cord of such an individual be severed, the properties of the resulting preparation are precisely those described in the last paragraph.

Transverse cuts through the dorsal third of the collar will include the collar nerve cord. Such an operation results in animals markedly less active than normal, resembling in this feature and in the greatly impaired shortening reflex animals with both trunk cords interrupted.

It is curiously difficult to observe a ganglionic or modifying function in the great cord of the collar which, from the anatomical evidence, has been thought to be a central organ. I have failed as yet to devise an experiment to demonstrate such a function. Its strategic and unusual position, along the path of the main reflexes, crucially affects its accessibility to experimental analysis. The great nerve centers of other animals are usually terminal, but here we are unable to isolate the ganglionic function without interrupting the main nerve paths that pass through the collar cord. It is perhaps significant that nothing more can at present be said than this: No effect of cutting the collar cord has revealed itself except such as can be explained as a parting of pathways. Thus burrowing and photic reactions are quite normal after collar cord section (or removal); but the shortening response of the trunk to proboscis stimulation is impaired in the same way that it is after interrupting the cords of the trunk.

To test the effect of the operations *per se*, aside from the interrup-

tion of nerve cords, I have made various mutilations of the body wall in regions not traversed by concentrations of nerve tissue. These have no detectable effect on the ability to jerk up the abdomen upon gentle proboscis stimulation. However, more drastic operations, such as removal of the anterior third or half of the proboscis, have a noticeable effect, at least temporarily. Such subjects show a decrease in spontaneous movement, especially exploratory progression, and are rarely stretched out straight, but remain coiled and quiet. These characteristics are relative; no profound change is found in the subjects in question. They show no loss in ability to respond normally to proboscis stimulation.

Section of the cords in isolated fragments of trunk region reveals a further detail of their function. Whereas the branchio-genital and
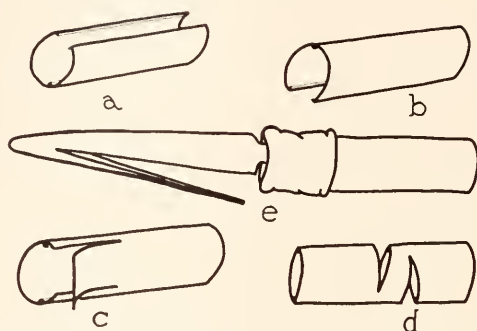

FIG. 2. Diagrams of operations on fragments of balanoglossids. See text for explanation.

especially the abdominal regions of intact animals exhibit almost continuous writhing movements, a length of trunk cut off from collar and proboscis lies motionless for many seconds or longer. Moreover, the occasional waves of contraction which do initiate in such pieces (especially branchial and terminal regions) or the slow peristaltic waves elicited by external stimuli are interrupted by interruption of the cords. If both cords are cut, only a few muscles immediately behind that level contract. If only one cord is cut, the waves are stopped, but not as sharply. Occasionally such a contraction wave starts up again after a delay of one half second or more and progresses to the level of the next cut. The ventral cord appears to be more influential, the dorsal somewhat less important, although the evidence for this difference is not here as clear as above.

A curious difference between dorsal and ventral nerve cords is revealed by the following experiment. This, further, anticipates the

next section by demonstrating that impulses are conducted through the lateral plexus, in this case between the cords in the transverse plane. A length of trunk region is opened by a mid-dorsal longitudinal slit (Fig. 2, *a*) and stimulated anywhere along the cut margin of one side. Moderate stimulation suffices to elicit not only a muscular response on the same side but also contralaterally.   An even weaker stimulus may produce an ipsilateral contraction alone.   The reciprocal preparation, however, opened by a mid-ventral slit (Fig. 2, *b*), will not, even upon strong stimulation, produce a contralateral response, although contractions of the same side are easily aroused.   These facts indicate some difference in the pattern of the nerve cords such that impulses readily cross the ventral but not the dorsal cord.   The ability to pass across the midline would seem, *a priori*, to be primitive; on this assumption the dorsal cord is more specialized than the ventral, although, as indicated by the previous experiments, the latter is the more important.

Such are the effects manifested in the first few hours after operating. What, now, happens to such operatives when kept, undisturbed, under favorable conditions, for some days?   Of a group of twelve specimens so treated, nine recovered normal ability to respond within ten days, four of these being normal by the fifth day.   Of the remaining three, two died and one recovered its active behavior but not its normal, rapid response.   Among the animals which recovered completely were subjects which had suffered transection of the collar cord and others whose dorsal and ventral nerve cords had been interrupted in the trunk. Complete tissue regeneration had not occurred, but, histologically, fibrous continuity of the cords was reestablished to a degree which was evidently sufficient to reestablish functional paths even in these short periods.

Experiments on the General Plexus

It has already been indicated that the body wall aside from the nerve cords is able to conduct nervous impulses.   This, it seems logical to assume, probably takes place through that thin basiepithelial nerve fiber layer which has been referred to as the general plexus.   The present section is devoted to an elucidation of its properties.

Diffuse Transmission.—The experiment with trunk segments slit open longitudinally demonstrated dorso-ventral conduction (actually involving an arc in the transverse plane).   By stimulating first one side and then the other of the first-described preparation, it can be seen that the lateral plexus is conducting now dorsally, now ventrally. The threshold of stimulation for conduction in this direction is, furthermore, low relative to that in the next several experiments.
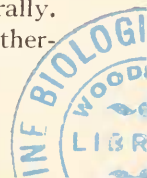
It was noted above that, after section of the great conduction tracts (collar cord or both trunk cords), persistent, strong stimulation of the proboscis will elicit contraction of the abdomen, although this contraction is delayed and abnormal in character. This suggests the existence of alternative, though poorer and slower paths. The general plexus can, therefore, conduct anteroposteriorly. In *Balanoglossus occidentalis* it was possible to demonstrate conduction anteriorly as well as posteriorly.

Such behavior is highly suggestive of a nerve net, for the property of diffuse transmission is characteristic of such nervous systems and is not found in others. The anatomical relations, as far as known, correspond with those of typical nerve net systems but the distinguishing features of the nerve net are more important physiologically than anatomically; moreover, the functional characteristics are more easily and certainly ascertained than the crucial histologic relations.

As illustrative of a number of other experiments demonstrating conduction through the lateral plexus, the following may be described. A long piece of trunk region is incised longitudinally just lateral to the dorsal cord and again just to one side—the same side—of the ventral cord, the incisions extending through about half of the length of the piece. There is thus produced a preparation (Fig. 2, *c*) from which projects a tongue of lateral body wall with no nerve cord, still attached, however, to the remainder of the trunk in which the cords are intact. The cordless tongue being now strongly stimulated, a delayed bilateral response in the uncut posterior end follows. This is much clearer in *B. occidentalis* than in *S. pusillus*, doubtless because the former's larger size permits a neater operation. In this species the response even extends forward into the unstimulated anterior region. Stimulation of this region, provided as it is with intact cords, produces prompt contraction of the posterior end, and need be only a fraction of the strength or duration of the stimulus required in the case of the cordless tongue. If the cords then be severed at the base of the tongue, the anterior region still possessed of cords will behave precisely as did the cordless tongue in respect to higher threshold and delayed conduction.

Again, a trunk fragment may be deeply notched, twice, transversely, one incision extending from the dorsal side through at least two-thirds of the animal's diameter, the other from the ventral side and about 5 mm. in front of or behind the first (Fig. 2, *d*). Strong stimulation anterior to the operations will elicit posterior responses, indicating the conduction of impulses around the overlapping obstacles, but with considerable difficulty. Occasional spontaneous contractions of these pieces were noticed. Beginning near one end

these spread as waves, apparently unimpeded by the cuts, to involve the whole fragment in contraction at the same time.

In all of these and other similar experiments it was noticed that much greater difficulty is encountered in anteroposterior transmission than in dorso-ventral. Much stronger stimuli are required for the former than for the latter.

The proboscis is especially favorable for demonstrations of diffuse transmission by reason of the absence of differentiated conduction paths. The proboscis may be slit nearly in two in any direction and yet respond as a whole to adequate stimulation of either half. This statement must be qualified since the proboscis does not respond to stimuli in the simple, certain manner of other parts, although it is the most sensitive region. It is as likely to continue its aimless motions as to react clearly to a prodding which suffices for the jerking up of the abdomen. The most dependable form of this experiment takes advantage of the fact that response of the posterior regions to proboscis stimulation is more reliable than its own response. The proboscis is cut obliquely, from the posterior margin toward a point on its main axis near the anterior end and in any plane about that axis (Fig. 2, *e*). The stimulus is then applied to the posterior end of the free tongue. The impulse is propagated anteriorly, around the cut, and then posteriorly to elicit a trunk shortening. Or the same experiment can be executed on preparations with the proboscis transversely and deeply notched, from any side, showing the absence of special conduction paths and the ability of the plexus to conduct impulses over whatever fraction of itself remains intact.

But the simplest demonstration that transmission may be truly diffuse utilizes fragments of isolated proboscis, thus eliminating any possible central nervous influence. Such fragments may be stimulated at any given point by a single contact stimulus and the response observed to take place all around this point and to involve a rapidly widening circle, i.e., impulses are being conducted in all directions away from this spot. Another stimulus at a neighboring point elicits the same behavior. But now some of the same paths are being traversed in the opposite direction. An infinity of different points may thus be stimulated with the same result and impulses shown to pass in both directions between any two of them.

Conduction with Decrement.— It is especially easy with the proboscis of *B. occidentalis* to demonstrate that the response to a stimulus spreads in every direction from the point of stimulation but only to a certain distance. Furthermore, this distance varies according to the strength of the stimulus, so that a larger area is called into muscular

contraction by a stronger stimulus. Again, we find a property characteristic of typical nerve nets (Parker, 1919) manifested in balanoglossids.

Autonomy.—The parts of the body of balanoglossids are remarkable for the degree of autonomy they exhibit. This property is highly significant from the point of view of the organization of the nervous system and we may describe its manifestations in isolated fragments of these animals.

Isolated proboscides immediately strike the attention by reason of their activity and irritability. They live well for many days. (I have never had opportunity to keep fragments long enough for any considerable amount of regeneration.) They respond to touch and to light as well as in the intact worm, move about in an exploratory manner—by means of cilia, and contract vigorously. But they are not as successful as are whole animals at burrowing, possibly for lack of weight and length in obtaining a purchase on the substratum. The most clearly autonomous major region, the proboscis, behaves alike attached or unattached to collar and trunk.

The isolated collar fragments which I have observed lived but one or two days before disintegrating. They were capable, however, of responding locally to touch and light. This region performs few muscular movements itself either in the intact animal or when isolated and most of its organs are of significance chiefly for the whole organism (collar cord, great blood vessels, buccal apparatus, specialized integumentary glands, skeleton). It has, nevertheless, the neuromuscular equipment to respond autonomously to stimuli.

Isolated trunks or parts thereof seem quite normal. Characteristically responding only by localized contraction of the spot stimulated, they exhibit generalized contractions only occasionally. If small enough, they progress by means of cilia; larger pieces do not progress at all. Sand is ingested and egested but burrowing is not effected. Longevity, in my specimens, was not as great as that of proboscis fragments, although Davydov (1908) and others have kept such fragments long enough for complete regeneration.

Thus, all regions possess the mechanism for nervous reflexes, and moreover, respond very largely by muscular contraction of very small areas immediately about the point of stimulation. It is not surprising, therefore, though crucially significant as an indication of the nervous pattern, to find that smaller and smaller fragments of the body wall are quite able to react nervously, the smallest piece that I could neatly cut and observe—having approximately 1 sq. mm. of surface—displaying nervous irritability and true reflexes. The evidences

against regarding this as direct muscular stimulation are: (1) the strength of adequate stimuli, which need be no greater than those required for indubitable, long distance reflexes in the intact animal whereas direct stimulation of muscles (thus in narcotized specimens) requires very strong stimuli, and (2) the reversible disappearance of the reflexes in light narcosis. Finally, it should be stated that fragments from any part of the surface of any region (except areas where the body wall is devoid of musculature) may thus react autonomously.

If small pieces of an animal, from any region, are able to execute normal reflexes, it is evident that the impulse does not need to travel to a distant central organ and then back to an effector, but that the entire path of the reflex is located within that small piece. Autonomy of this type is highly characteristic of nerve net-equipped organisms and its presence in balanoglossids is convincing evidence that the general superficial plexus of these animals is essentially a nerve net.

Polarization.—One of the earliest modifications of the primitive nerve net was in the direction of acquiring polarity. This means that impulses will travel more easily in one direction than in others, and will tend to spread differentially along an axis instead of uniformly in an expanding circle. It is of interest to look for evidence of polarity in the diffuse plexus of enteropneusts.

A proboscis cut into two pieces, transversely, exhibits no difference in the behavior of the two cut faces. The pieces themselves show no evidence of polarity aside from the direction of progression which is a result of the polarity of the ciliated field. It has already been shown that impulses spread in all directions from the point of stimulation in the proboscis.

The trunk exhibits an obvious polarity traceable to the nerve cords, but in the absence of these structures the lateral plexus shows little evidence thereof. Evidence has already been cited, however, which indicates a differential orientation of pathways such that impulses pass easily in the dorso-ventral direction, but only with difficulty longitudinally. No experiments have been devised to show that these two axes are heteropolar, although it is to be expected from other, already cited facts. The poles of the dorso-ventral axis would be the dorsal and ventral cords. These differ in several ways, not only histologically, but functionally, thus in respect to passage of impulses across the midline and in relative importance for the normal responses of the intact animal. Along the anteroposterior axis the usual direction of propagation of waves of contraction is from anterior to posterior. How much this normally depends on the cords cannot yet be

said, but it is possible that a degree of anteroposterior polarity exists in the plexus.

Action of Drugs

Unfortunately, almost no opportunity has been found to experiment with drugs whose effect on the nervous systems of higher animals is known. But two observations may be mentioned as of some potential interest.

The anaesthetic action of $MgSO_4$, chloretone, and cocaine has been frequently employed in other connections. The sequence of events in this action is suggestive of a distinction between two categories of nervous mechanisms. Whole specimens or fragments were exposed to these agents and tested from time to time for their responses to mechanical stimulation. It was found that local responses of the area immediately about the spot stimulated persisted long after the cessation of more general ones, involving major conduction paths.

The only other drug whose action on the nervous system has been tested is strychnine. Parker (1919), discussing some experiments of Moore (1917), suggests that this drug might afford a physiological test for the presence of synapses. Moore had found that strychnine exerted no effect on certain coelenterates, a slight effect on some echinoderms, and a considerable one on certain crustacea and cephalopods, a series which leads nicely up to the well-known effect on vertebrates generally regarded as a heightening of irritability by decrease of synaptic resistance. Whole specimens of *Saccoglossus pusillus* and pieces of *Balanoglossus occidentalis*, immersed for periods up to several hours in concentrations of from 1 : 1000 to 1 : 100,000 of strychnine sulphate in sea water, showed no effect whatsoever. The responses appeared entirely normal in character, strength, and threshold of stimulation.

This experiment points to a condition similar to that of coelenterates and different from that of higher organisms. But, since the existence of synapses in coelenterates, long denied, has recently been claimed (Bozler, 1927; Hanström, 1928; Woollard and Harpman, 1939), this physiological difference cannot be safely interpreted as proving anatomical continuity. Nor can I follow Parker (1919) and Ariëns Kappers (1929) in assuming that the existence of a physiological nerve net, evidenced by the properties of diffuse transmission, autonomy, etc., necessarily means protoplasmic continuity of neurons. At present it cannot be said whether the physiological nerve net of balanoglossids is synaptic or asynaptic.

Summary

1. A picture of the functional plan of organization of the nervous system of balanoglossids, based on experiments with *Saccoglossus pusillus* and *Balanoglossus occidentalis*, is offered. The state of the receptor and effector mechanisms is also reviewed and correlated with the habit of life.

2. These animals are shown to be highly dependent on a general superficial ciliature whose complex behavior suggests the possibility of nervous control.

3. A great abundance of several types of integumentary glands capable of voluminous secretion, represents a second important effector system. Lacking any physiological evidence of the nature of the products and the variations in activity of the glands, nothing can yet be said regarding the relation of this apparatus to the nervous system.

4. Identification of the organs of light production has not been made. Crozier's argument for their independence from the nervous system is controverted but no positive statement of their nature and control can yet be made.

5. The muscular system is characterized by sluggish action and produces movements of a simple generalized character, lacking differentiation into a variety of reflexes. The paucity of clearly defined and diversified responses greatly limits the possibilities and conditions the nature of experiments on the nervous system.

6. The receptors, likewise, are at a low level of development, lower than that of many coelenterates, being represented by generalized, scattered sense cells. The mechanism of the photic response is discussed in the light of new experiments.

7. The nervous system is shown experimentally to be diffuse and superficial.

8. Properties characteristic of nerve nets—diffuse transmission, decremental conduction, and neuromuscular autonomy of every small fragment of body wall—are demonstrated to exist in balanoglossids. But the presumption of anatomical continuity of neurons from these physiological properties is not assumed, though the facts point to relations resembling those of coelenterates and differing from those of higher animals.

9. The physiological nerve net has been modified correlative with the elongated, bilaterally symmetrical body and the development of great conduction paths in the mid-dorsal and mid-ventral lines. Conduction through the general plexus is more easily induced in the transverse plane than longitudinally.

10. Specialized conduction tracts are demonstrated which correspond to the known histologic concentrations of punctate tissue.

11. Of these, the ventral tract in the trunk is less dispensable than the dorsal, but the latter may be slightly more specialized.

12. Almost no suggestions have been found that these cords, including the internal nerve cord of the collar, function in a ganglionic or central nervous capacity. They are all primarily conduction paths.

13. These facts, together with the general behavior of the animals and the high degree of autonomy of parts are considered to render inappropriate the designation "central nervous system" for the collar nerve cord or any or all of the concentrations of nerve tissue in balanoglossids.

14. The picture of the nervous mechanism of the Enteropneusta is that of an exceedingly primitively organized system. This must be emphasized, but is not considered to affect, one way or the other, the morphologic arguments concerning the relations of these animals and the chordates.

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